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Identification of the Constituent of Non-Triglyceride Components of Jatropha Seed Oil

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Abstract

Vegetable oils are mostly triglyceride of saturated and unsaturated fatty acid, but they also contain a small percentage of non- triglyceride components that belong to wide chemical classes and could contribute to the nutritive value or anti-nutritional value of the vegetable oil. Jatropha oil is non edible because it is considered to contain phorbol ester, a toxin and a tumour promoter that is found in jatropha seeds. This research was carried out with the aim of identifying the content of the volatile component of jatropha oil. Jatropha seeds were extracted with n-hexane using soxhlet extraction method and quality parameters of iodine value, acid value and peroxide value were determined. The Fourier Transform-Infrared spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were carried out in order to identify the volatile chemical compounds in the oil. The oil yield was 43.17%, and the iodine value, acid value and peroxide values were 100 mgI_2/100 g, 2.39 mgKOH/g, and 8.36 meq/kg respectively. The FT-IR analysis revealed the presence of compounds with the functional groups of alkanes, alkenes, alcohols, esters carboxylic acid, amine and carbonyl. The GC-MS analysis showed the presence of twenty compounds of different chemical classes. It was concluded that jatropha seed oil contains many different types of compounds in addition to triglycerides.

Keywords; Jatropha, non-triglycerides, oil, analysis, chromatogram, spectrum

INTRODUCTION

Jatropha (also known as Bita da zugu in Hausa language) seed oil gotten from Jatropha seeds. Jatropha seeds and oil with the exception of the Mexican variety, are not edible due to the presence of some toxins mostly phorbol ester (phorbol 12-myritate acetate) and curcin (Li *et al*., 2010; Kaushik *et al*., 2010) and for this reason Jatropha seed oil could be used for making non edible products since it is not competed for by food industry. Jatropha seed oil has 77 % to 84 % unsaturated acids depending on the variety and region, (Haruna and Idris, 2018), and has the iodine value of about 103 gI_2 /100 g (Emil *et al*., 2009).

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Table 1: Percentage Fatty Acid Composition of *Jatropha curcas* Seed Oil

Fatty acid	% Composition
Lauric acid	0.019
Palmitic acid	14.24
Palmitoleic acid	10.16
Stearic acid	5.15
Oleic	52.27
Linoleic	27.87
Linolenic	0.29

Source: Ugbogu *et al*.,(2014)

Jatropha oil, like any other fixed vegetable oil is made up of mostly triglycerides (Karmakar et al., 2020), but they also contain a small proportion of non-triglyceride (volatile) matter (Narasinga Rao 2001), most of which are extracted with the oil from seeds. The volatile component of jatropha oil would be of diverse organic classes, including poisonous phorbol ester that are known to be in jatropha seeds and oil (Li *et al*., 2010). Most of the volatile chemicals in jatropha seed oil are oil soluble and are usually extracted with the oil during oil extraction while others are degradation products of the triglycerides. All these volatile chemicals constitute part of the volatile component of the vegetable oil, and are very important because they determine the use of the oil. This component of the vegetable oil defines the colour, taste, flavour, and aroma of the vegetable oil as well as its physiological characteristics. The classification of vegetable oils as edible and non-edible oils depends mostly on the type and the amount of non-triglyceride components of the vegetable oil (Menezes et al., 2006, Sharma and Singh 2008). Jatropha seed oil is one of the vegetable oils classified as nonedible because of it poisonous phorbol ester expected to be extracted with the oil from the jatropha seeds. The knowledge of compounds that constitute the volatile components of vegetable oils is therefore important for the classification of vegetable oils. This world production of vegetable oil is expected to increase as demand increases since there is enough farmland and improved technology (Sarker, 2023). Nowadays the chemical industry has developed a number of new processes for the chemical conversion of vegetable oils into various valuable industrial raw material and products (Gujar, & Modhera, 2023). This research is aimed at determining the constituent of the volatile component of the jatropha seed oil.

MATERIALS AND METHODS

Materials

The materials used for this research are jatroph seeds, n-hexane and methanol. The chemicals used were of analytical grade. The equipment used were FT-IR, GC-MS machines, soxhlet extractor and ordinary laboratory instruments.

Methods

Collection and Identification of seeds.

The jatropha plant from where the seeds were harvested were identified growing in the botanical garden of Gombe State University by a botanist in Gombe State University.

Processing of seeds.

The Jatropha seed pods were picked from the botanical garden of the Biology Department of Gombe State University, and were dried and hand cracked. The cracked seeds were sorted, sun dried, and oven dried at the temperatures of 40 ^{o}C for 24 hours. The dried seeds were crushed with a corn miller, and kept in dry polythene bags for oil extraction.

Oil Extraction

The oil was extracted using Soxhlet extraction method with n-hexane as the solvent in the chemistry laboratory of Gombe State University. A 50 g of the crushed jatropha seeds was weighed into the extraction thimble, put into the extractor and was inserted into a weighed 500 cm^3 round bottom flask which was half filled with n-hexane and was mounted on a heating mantle. The condenser was connected and the water was allowed to run through the condenser as the power was switched on. The extraction was allowed for about 8hours when there was no sign of oil in the seed cake. The extractor was replaced with the Liebig condenser and the solvent distilled off and recovered from the oil. The flask containing the solvent free oil was reweighed and recorded (Fai *et al*., 2023).

Calculation

$$\% \text{ Oil yield} = \frac{(W2-W1)x100}{\text{Weight of Sample (cm3)}}$$

Where:

W_2 = weight of flask and oil

W_1 = weight of empty flask

Preparation of Reagents.

1. 0.1 M NaOH: 4 g of sodium hydroxide was weighed and dissolved in 1000 cm^3 of distilled water.
2. Phenolphthalein indicator: 0.1 g of Phenolphthalein was weighed and dissolved in 50 cm^3 ethanol and then 50ml of distilled water was added in a 1000 cm^3 conical flask. This was then made up to the mark with boiled distilled water.
3. Starch indicator: 1 g of soluble starch was weighed and dissolved in 50cm^3 distilled water in a 250 cm^3 conical flask. This was then transmitted into the mark with boiled distilled water.
4. 10 % aqueous potassium iodine: 10 g of potassium iodine was weighed and dissolved into 20ml of distilled water in a 250 cm^3 beaker. It was then transferred in 1000 cm^3 volumetric flask and distilled water was added up to the mark.
5. Wij's reagent: 7.8 g of iodine trichloride and 8.5 g of iodine were dissolved in glacial acetic acid in separate flasks and warned. After cooling, it was then transferred both into a 1000 cm3 volumetric flask and make up to mark with glacial acetic acid.
6. 0.5M hydrochloric acid: this was prepared using the formula:

$$\mathbf{C_1V_1 = C_2V_2}$$

$$\mathbf{C_1} = \frac{SG \times \%\ PURITY \times 10}{MW}$$

$$= \frac{1.19 \times 37\ 10}{36.5}$$

$$= 12.06M$$

$$\mathbf{C_1V_1 = C_2V_2}$$

$$\text{Where: } V_1 = \frac{C_2V_2}{C_1}$$

$$V_1 = \frac{0.5 \times 100}{M_1}$$

$$V_1 = \frac{0.5 \times 100}{12.06}$$

$$V_1 = 4.145\ cm^3$$

Where C = concentration of HCl = 0.5 M

MW = molecular weight of acid = 36.5 g

V = volume needed = 100 cm^3

SG = specific gravity = 1.19

% purity = percentage purity = 37 %

Therefore, 4.145 cm^3 of HCl was taken into 100 cm^3 volumetric flask and dilute to mark with distilled water.0.5 cm^3 sodium thiosulphate: A 4 g of sodium thiosulphate ($Na_2S_2O_3$) was weighed and dissolved in 1000 cm^3 of distilled water. It was then transferred into 1000 cm^3 volumetric flask.

0.5 M alcoholic KOH: A 28 g of KOH was weighed and dissolved in 20 cm^3 of distilled water in a 25 cm^3 conical flask. It was then transferred into 1000 cm^3 volumetric flask and make up to the mark with absolute alcohol (ethanol).

Characterization of Vegetable Oil Samples

Determination of iodine value. Official Method of Analysis (A.O.A.C.) (2023) was employed with little modification. A 0.2 cm^3 of oil sample was weighed into a 250 cm^3 conical flask and dissolved with 15 cm^3 carbon tetrachloride, and 25 cm^3 of Wijs reagent was added to the mixture. The flask was then stoppered and gently shaken and placed in the dark for 30 minutes. The excess iodine was determined by adding 20 cm^3 10 % (W/V) potassium iodine solution and 150 cm^3 water and titrating this with 0.1 M sodium thiosulphate using starch as indicator. The titration was continued until blue colour just disappeared after a vigorous shaking. A blank determination was carried out and the iodine value (IV) was determined. The procedure was repeated **(Fai *et al.*, 2023).**

Calculation

The iodine value of oil sample is determined using the equation below;

$$\text{Iodine Value (g } I_2 \text{ / 100 g oil)} = \frac{12.69 \times C \times (V1 - V2)}{\text{Weight of Sample (cm3)}}$$

Determination of acid value (A.O.A.C.)

A 5.0 cm^3 of oil sample was weighed into a 250 cm^3 conical flask, 50 cm^3 solvent mixtures (1:1) of 95 % ethanol and diethyl ether were added and the solution was titrated with 0.1M potassium hydroxide using 1cm^3 of 1 % (W/V) of phenolphthalein as indicator until pink coloration persisted. The acid value was computed from the expression;

$$\text{Acid Value (mg KOH/ g oil)} = \frac{56.1 \times C \times V}{\text{Weight of Sample (cm3)}}$$

Determination of Peroxide Value (A.O.A.C.)

A 0.5 g of oil was weighed into 250 cm^3 conical flask. 10 cm^3 of chloroform and 15 cm^3 of acetic acid were added and the mixture stirred after which 1 cm^3 of (10% W/V) potassium iodide was added. The flask was stopped and shaken for 1 minute. The flask was placed in the dark for 5 minutes and 75cm^3 of water was added. The iodine liberated was titrated with standard (0.1M) sodium thiosulphate until yellow colour was almost gone.

Blank determination was conducted. The peroxide value (PV) was computed from the relationship:

$$\text{Peroxide Value (mg } O_2\text{/g oil)} = \frac{(V0 - V) \times C}{\text{Weight of Sample (cm3)}}$$

Spectroscopic Analysis of Vegetable Oil.

IR analysis.

The IR analysis of the oil was carried out in the Biochemistry laboratory of the Gombe State University using IR machine type' Perkin Elmer Spectrum Version 10.03.09'

Gas chromatography-mass spectrometer (GC-MS) analysis

The GC-MS analysis of the Jatropha seed oil was carried out in the American University of Nigeria (AUN) Adamawa. The gas chromatography-mass spectrometer model; 'GC-MS-7890A, Agilant Technology Inert MSD-597CM' was used with column of agilant-1 fused silica; capillary column (30 m x 250 µm x 0.25 µm, composed of 5 % Phenyl Methyl Silox). For GC-MS detection, an electron ionization system with ionizing energy of 74 eV was used. Helium gas was used as the carrier gas at constant flow rate of 3.8379 cm^3/min and an injection volume of 1µl was employed with split-less injection mode, injection temperature of 270 ^{0}C and ion source temperature 250 ^{0}C. The oven temperature was programed initially at 80 ^{0}C for 0 minute, decrease by 10 ^{0}C for 1 minute then increased to 300 ^{0}C for 5 minutes. The flow control mode was at an average velocity of 72.418 cm/sec, pressure 32.475 psi, the column flow was 3.8379 cm^3/minute, and the purge flow was 1 ml/minute. The total flow was 54.838 cm^3/minutes. Mass spectra were taken at 74 eV, a scan of 27 minutes and fragment from 50 g to550 g.

RESULTS AND DISCUSSION

Oil Quality Parameters

The oil quality parameters of Jatropha, seed oil are shown on Table 1. The Soxhlet extraction method yielded 43.17% jatropha oil. This yield is lower than the yield of 46.31% reported by Nayak and Patel (2010) and also lower than the range of 50-60% (Iqbal 2015) for different jatropha oils extracted using the same method from different jatropha varieties grown in Bangabadhu Seikh Mujibuj Rahman Agricultural University, but within the yield range of 38.7-45.8% (Mbako et al 2020) for jatropha oil extracted at different maturity stages of the seeds in Bardeoli. The iodine value of vegetable oils indicate the level of unsaturation of the oil. The iodine values of jatropha was found to be 100 mgI_2 /100 g. The iodine values of jatopha (100 mgI_2 /100 g) is lower than 125 mgI_2 /100 g and as such it is a non-drying oils (Wikipedia). The iodine value (100 mgI_2 /100 g) of jatropha seed oil is lower than the value (103 mgI_2 /100 g) obtained by Emil *et al*., (2009) and 108.92 mgI_2 /100 g obtained by Adama (2021). However, the differences are not much and could be because of errors from instrument. The differences could also be due to geographical location and soil variability. Acid value indicates the amount of free fatty acid in the oil. A high acid value indicates the level of deterioration by hydrolysis and rancidity. Acid value indicates the amount of free fatty acid in the oil. A high acid value indicates the level of deterioration by hydrolysis and also rancidity. The acid value of jatropha oil was found to be 2.39 mgKOH/g. This acid value of 2.39 mgKOH/g is higher than the value of 0.86 mgKOH/g obtained by Rokhsana *et al*., (2018) and 0.988 mgKOH/g of Adama (2021) for jatropha oil. This could be because of variability in soil and other environmental conditions.

The peroxides values of jatropha oil was 8.36 meq/kg meq/kg. The peroxide value indicates the level of active oxygen in the vegetable oil and the value is related to storage or exposure to the environmental oxygen. A high peroxide value means the oil was not well stored or preserved and also indicates rancidity of the oil. When the peroxide values of the oils is less than ten milligram equivalent it implies that the oil is very good (not rancid) and when it is above ten milligram equivalent it means the oil is still good (Gordon 2001). The peroxide value of 8.36 meq/kg for jatropha oil is higher that the value of 6.83 meq/kg obtained by Adama (2021).

The differences could be because of the differences in storage of the seed or oil.

Table 2: Oil quality parameters of jatropha oil

Vegetable oils	Iodine value (gI_2/100g)	Acid value (mg KOH/g of oil)	Peroxide value (meq/kg)	Percentage yield (%)
Jatropha oil	100	2.39	8.36	43.17

IR Results of Jatropha Oil

The IR results for jatropha oil are presented on Figure1 and Table 3. The absorption peaks are at around 3600 cm^{-1}, 340 cm^{-1} -2400 cm^{-1}, 2850 cm^{-1},1740 cm^{-1},1320 cm^{-1}, 1200 cm^{-1},1300 cm^{-1} - 1000 cm^{-1}, 3007 cm^{-1} asymetric and symmetric aldehydes at, 1400 cm^{-1}, 1600 cm^{-1}, 1018 cm^{-1} and 720 cm. All the peaks indicate the presence of alkanes, alkenes, alcohols, carboxylic acids, ketone, aldehydes and esters (table 3). The chromatogram is similar to that of njangsa oil (Fai et al 2023), and as such contain almost same functional groups as in njangsa oil in Fai et al (2023). However the intensities of the peaks are not the same. Njangsa oil transmittance is 25 % at 3423 cm^{-1} in Fai et al (2023), while that of jatropha oil is 55 % at 3423 cm^{-1}, implying that the functional group absorbing at 3423 cm^{-1} is of a higher concentration (Othman 2022) in njangsa oil.

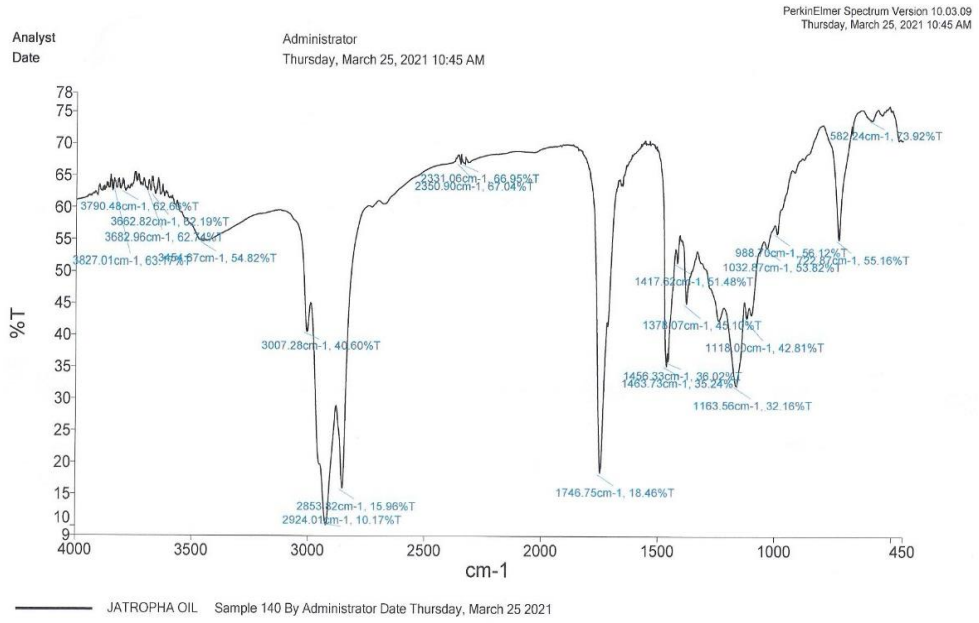

Figure 1 : IR Spectrum of Jatropha Oil

Table 3. IR Revealed functional group in Jatropha Oil

Vibrational frequency (cm^{-1})	Functional group	Remark	Conclusion
Strong and sharp peak at 1746.75 cm^{-1}	-C=O	Carbonyl group	Compounds containing Ketone, alkane, alkene esters, carboxylic acids and hydroxy functional groups are present
Weak sharp peak at 3007.28 cm^{-1}	Vinyl C-H stretch	Alkene group	
Two peaks at 2853.82 cm^{-1} and 2924.01 cm^{-1}	Alkyl SP^3 C-H stretch	Alkane	
Weak and broad peak at 3454.6 cm^{-1}	-OH stretch	Alcohol	

The IR chromatogram of Jatropha seed oil is similar to that obtained by Alpandi *et al*., (2022) in which he suggested, the presence of alcohols, alkanes, alkenes, esters tertiary and secondary alcohols, and phenols. However, unlike this chromatogram he had no carboxylic acids,

ketones and aldehydes in his assessment of his chromatogram of the Malaysian Jatropha seed oil. This could be because of differences in oil extraction processes.
The chromatogram of jatropha oil differs from that of Mubarak (2014) jatropha oil in the absence of absorption peaks at above 3008.24 cm^{-1}, and at 2350 cm^{-1} ,2331 cm^{-1} , 1456 cm^{-1}, and 1032 cm^{-1} this could be because of the type of jatropha oil he used.

GC Result of Jatropha Oil

The result of GC-MS analysis of jatropha oil are presented on Figure2, and Table4. The chromatogram for jatropha oil presented on figure 6 has many peaks. However, some of the peaks represent the same compound on the spectra. Twenty different volatile compounds were identified in jatropha oil (Table 4) unlike twenty-four non-triglyceride compound found in njangsa seed oil (Fai et al 2023) . The compound found in jatropha seed oil are of different classes which include; hydrocarbon of different kinds, long chain fatty acids, esters and, aldehydes, alcohols, ethers, and epoxides. Jatropa oil has more different types of fatty acids than njangsa oil (Fai et al 2023). The presence of different types of acid in jatropha oil (table 4) justifies the higher acid value (2.39 meqKOH/g) of jatropha oil (Table 2).

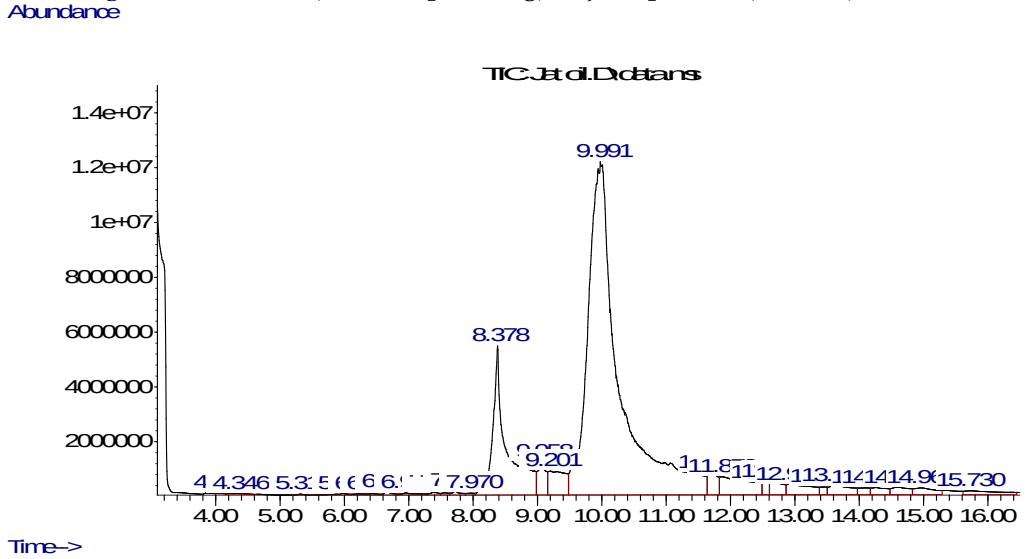

Figure 2: GC Chromatogram of Jatopha Oil

Table 4: List of compounds in jatropha oil

Compound	Formula
Cyclopropane, nonyl-	$C_{12}H_{24}$
Cis-4-decenal	$C_{10}H_{18}O$
1-decanal,2-octyl	$C_{18}H_{38}O$
Carbonic acid, dodecyl vinyl ester	$C_{15}H_{28}O_3$
Heptyl, octacosyl ester	$C_{15}H_{32}O$
Oxalic acid cyclobutylheptadecylester	$C_{23}H_{42}O_4$
1-decanol,2-octyl	$C_{18}H_{38}O$
Hexadecanoic acid meythyl ester	$C_{17}H_{34}O_4$
Oxirane((dodecyloxy) methyl)	$C_{15}H_{30}O_2$
n-hexadecanoic acid	$C_{16}H_{32}O_2$
Oleic acid	$C_{18}H_{34}O_2$
9-tetradecenal(Z)	$C_{14}H_{26}O$
Cis-Vaccenoic acid	$C_{18}H_{34}O_2$
1-hexacosanol	$C_{26}H_{54}O$
9-Eicosenoic acid	$C_{20}H_{38}O_2$
9,12-octadecadienal	$C_{18}H_{32}O$
n-tracosanol-1	$C_{24}H_{50}O$
Heptadecanoic acid heptadecyl ester	$C_{34}H_{64}O_2$
2,6-octadien-1-ol,3,7-dimethyl (Z)	$C_{10}H_{18}O$

The presence of different classes of organic compounds in jatropha oil (table 4) justifies the presence of different functional groups in IR results of jatropha oil (Table 3). The presence of different fatty acids such as oleic acid, n-hexadecanoic acid, 9-eicosenoic acid and cis-vaccinoic acid agrees with the fact that the acid value of the oil was not zero (table2). It could be observed that all the non-triglyceride compounds of this jatropha oil contain oxygen, except one (cyclopropane, nonyl-) which is a hydrocarbon unlike njangsa seed oil in Fai et al (2023) that contain different types of hydrocarbon including terpenes. However the absence of terpenes, tocols and phenolics in jatropha oil (a non-edible oil) is in line with the findings of Dhara et al (2010) and Tian et al (2023) that they are found mostly in common edible oils.

CONCLUSION

Vegetable oil was extracted from seeds of jatropha plant with n-hexane using normal soxhlet extraction method. The extraction gave appreciable yield of above 37 %, implying that the seed could be classified as oil seeds. The other quality parameters analyzed indicated that the oil was of good quantity. The spectroscopic analysis of the oil revealed the presence of twenty volatile compounds with different organic functional groups. It could therefore be concluded that jatropha, seeds oil contains different types of volatile compounds in addition to their triglyceride compounds.

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